RELM-beta (human) Matched Pair Detection Set

[Resistin-like molecule-beta (human) Matched Pair Detection Set]

Cat. N° AG-46A-0001-KI01 For Research Use Only 17-February-2015

1. Intended Use

The RELM-beta (human) Matched Pair Detection Set contains key reagents required to develop sandwich ELISA to measure human RELM-beta.

2. Materials Provided

• AG-46A-0001/STD	Standard	1 vial (1 µg) (lyophilized)	(STD)
• AG-46A-0001/COAT	Coating Antibody	1 vial (300 µl)	(COAT)
• AG-46A-0001/DET	Detection Antibody	1 vial (120 µl)	(DET)
• AG-46A-0001/HRP	Anti-Rabbit IgG-HRP	1 vial (8 µg) (lyophilized)	(HRP)

3. Materials Required

- PBS: 137mM NaCl, 2.7mM KCl, 8.1mM Na_2HPO_4, 1.5mM KH_2PO_4, pH 7.2, 0.2µM filtered
- Wash Buffer: 0.1% Tween® 20 in PBS
- ELISA Buffer: 0.2% BSA (ELISA grade) and 0.05% Tween®20 in PBS
- Coating Buffer: PBS
- Blocking Buffer: 2% BSA (ELISA grade) in PBS, 0.2 µm filtered
- TMB: tetramethylbenzidine
- Plates: Nunc-Immuno MaxiSorp, N°467466
- Stop Solution: 2M sulphuric acid (H_2SO_4)
- Distilled or deionized water (DW)

4. Product Specifications

- Number of Assays: Contains sufficient materials to run ELISAs on 5 x 96-well plates.
- Range: 0.016 ng/ml to 1 ng/ml
- Specificity: Recognizes human RELM-beta.
- Stability: Stable at least 1 year after receipt when stored at -20°C.
  For long term storage, aliquot the STD and keep at -20°C.

5. General ELISA Protocol

a) Reagent Preparation

- Centrifuge the vials before opening to recover entire contents of the vial.

- Dilute the desired amount of **Coating Antibody (COAT)** (1 mg/ml) (mouse monoclonal antibody) to 5 µg/ml in Coating Buffer and use it fresh.

- Dilute the desired amount of **Detection Antibody (DET)** (1 mg/ml) (rabbit polyclonal antibody) to to 2 µg/ml in ELISA Buffer and use it fresh.

- Reconstitute the **Anti-rabbit IgG-HRP (HRP)** with 800 µl DW. After reconstitution, prepare aliquots and store the reconstituted HRP at 2-8°C. Dilute the reconstituted Anti-rabbit IgG-HRP to the working concentration by adding 120 µl in 12 ml of ELISA Buffer (1:100).

- Reconstitute **the Standard (STD)** (recombinant human RELM-beta) with 100 µl DW to obtain a concentration of 10 µg/ml. After reconstitution, prepare aliquots and store the reconstituted standard at -20°C. **Avoid freeze/ thaw cycles**. A standard curve using 2-fold serial dilutions in ELISA Buffer is recommended. Suggested standard points are 1 ng/ml, 0.5 ng/ml, 0.25 ng/ml, 0.125 ng/ml, 0.063 ng/ml, 0.031 ng/ml, 0.016 ng/ml and 0 ng/ml.

Note that diluted reagents described in this section are not stable and cannot be stored.

b) Plate Preparation

1. Coat the wells by adding 100 µl to each well of diluted Coating Antibody (5 µg/ml) (see section Reagent Preparation) to a 96-well ELISA microplate (Nunc-Immuno MaxiSorp, N°467466 is suggested). Cover the plate with plate sealer and leave overnight at 4°C.
2. Aspirate the coated wells. Remove any remaining liquid by inverting the plate and blotting it against clean absorbent paper.
3. Block plates by adding 200 µl of Blocking Buffer for 2 hours at room temperature (RT).
4. Aspirate the coated wells and add 300 µl of Wash Buffer using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by inverting the plate and blotting it against clean absorbent paper.

c) Assay Procedure

1. Add a total of 100 µl to each well of analytes (diluted in ELISA Buffer) or standard (see section Reagent Preparation) to the plate.
2. Cover the plate with plate sealer and incubate for 3 hours at room temperature.
3. Repeat the aspiration/wash as in step 4 of **Plate Preparation**.
4. Add 100 µl to each well of the diluted Detection Antibody (2 µg/ml) (see section **Reagent Preparation**).
5. Cover the plate with plate sealer and incubate for 1 hour at room temperature.
6. Repeat the aspiration/wash as in step 4 of **Plate Preparation**.
7. Add 100 µl to each well of the diluted Anti-rabbit IgG-HRP (see section **Reagent Preparation**).
8. Cover the plate with plate sealer and incubate for 30 min at room temperature.
9. Repeat the aspiration/wash as in step 4 of **Plate Preparation**.
10. Substrate development is conducted by addition of 100 µl to each well of ready-to-use TMB for 20-30 min at room temperature.
11. Stop the reaction by adding 100 µl of Stop Solution (2 M H_2SO_4). Tap the plate gently to ensure thorough mixing.
12. Measure the OD at 450 nm in an ELISA reader.
13. Measure absorbance at 550 nm and subtract these values from those obtained at 450 nm to correct for optical imperfections in the microplate. If absorbance at 550 nm is not possible, measure the absorbance at 450 nm only.
    Note: When the 550 nm measurement is omitted, absorbance values will be higher.

6. Technical Hints

- Once reagents have been added to the plate, DO NOT let the plate dry at any time during the assay.
- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and equipment. Automated plate washers can become contaminated thereby causing assay variability. Buffers containing a large quantity of proteins should be made under sterile conditions and stored at 2-8°C or be prepared fresh daily.
- Vigorous plate washing is essential.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedure.
- The Stop Solution (STOP) consists of sulphuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

7. Calculation of Results

- Average the duplicate readings for each standard and sample and subtract the average blank value.
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the horizontal (X) axis vs. the corresponding RELM-beta concentration (ng/ml) on the vertical (Y) axis (see **8. Expected Standard Curve**).
- Calculate the RELM-beta concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation.
- If the test samples were diluted, multiply the interpolated values by the dilution factor to calculate ng/ml of human RELM-beta in the samples.

8. Expected Standard Curve

The following curve is obtained using the different concentrations of standard as described in this protocol:

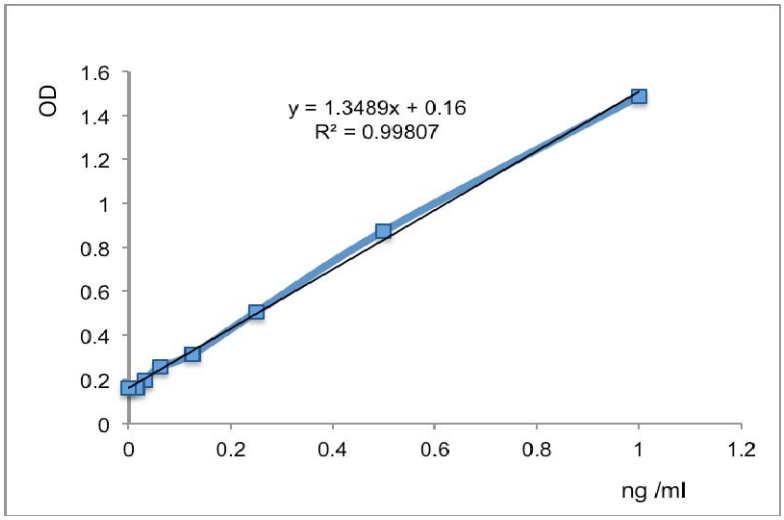

Figure: Standard Curve

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